HARVARD MEDICAL SCHOOL
DEPARTMENT OF BIOLOGICAL CHEMISTRY
BOSTON, MASSACHUSETTS 02115

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Dear Don,

I want to thank you for your very kind help in my search for a job. I think I'll be satisfied if things work out either at Boulder or Santa Barbara, but I'm continuing to follow up other possibilities as well. In any event it seems only fair to bring you up to date on my experiences as a neurobiologist, especially since developments over the past year have brought the project out of the doldrums of frustration into a period of gratifying and even occasionally exciting progress.

First, we (a technician working with me and I) have evolved a set of techniques that provide very satisfactory cultures of at least one type of neuron - those from chick embryo dorsal root ganglia. The cultures are essentially free of fibroblasts and satellite cells so that truly "isolated" neurons are available for study. These neurons can retain a healthy appearance for over a month, during which time they extend long, arborating processes, form complex-looking "networks" with neighboring cells, and pass through the characteristic stages of cyton development to assume a relatively mature morphological appearance. (A photo of a 22 day "old" neuron is enclosed.) We have applied the same techniques to mouse embryo DRG cells with somewhat less but encouraging success. (You may remember that I am partial to the mouse as a cell source for the long run.)

Second, I've found a quite easy way to evoke and record action potentials extracellularly from the processes of the cultured cells. The method (which has worked with both chick and mouse cells) is little more than a classical one - nerves lifted into oil on metal electrodes - adapted to a micro-scale. It isn't the sought after ideal applicable to long term studies since even though the cells involved frequently remain viable, the processes rarely escape damage, and it doesn't employ the "microelectrode array" which absorbed so much of my early effort. But the technique has permitted some immediate results of interest, among them a clear demonstration that the processes extended by isolated neurons in culture <u>are</u> capable of propagating action potentials, a partial investigation into how soon after outgrowth (i.e., how near the growing tip) this capability is expressed, measurements of propagation rates (with multiple - electrode arrangements), and some exploration of the "complex" activity (patterned spike trains) that is found especially in thick processes (probably fiber bundles, or "fascicles" as some call them) of older cultures. The method

should also be good enough for an attack on some more advanced sorts of question, such as signal processing at branch points, long term effects of excitation on cell bodies, and, the jackpot of them all - the existence of synapses. At present, I'm setting up the auxiliary apparatus that I hope will permit biochemical tests for chemically mediated synapses in the sensory cell cultures. Also, to cover our bets, we're learning to culture cell mixtures (sensory + spinal cord + muscle) in which there is some *a priori* reason to expect synapses.

The microelectrode array which was the original focus of the project and the motivation for Charlie Thomas' entry into neurobiology has been set aside for the while. But both Charlie and I are trying to use what we now know about requirements for extracellular recording from cultured neurons to think of ways to make the array concept workable. (In the original design, the array electrodes were being exposed only to the extraordinarily small, noise-masked potentials available even very close to single cells or processes bathed in low-impedance culture medium. Since we now know the potentials are really there and know at least one way to record them extracellularly, a successful revision of the array design seems ultimately quite likely.)

One other development worth mentioning is the addition of autoradiography to the system. Two graduate students working with me part time on a voluntary basis (first year "research exploration") have had very impressive success in this line, and we now have quite easy assays for synthetic activities at the single cell level with some useful sub-cellular resolution. Right now, we're using the assays in simple ways; e.g., to test the time course, completeness, and reversibility of metabolic inhibitors that may be useful in the future; to look at protein transport rates and the possible deposition of protein during process growth; and to test for UV-induced DNA repair synthesis. But the assays should be even more valuable later on.

I hope all the above gives you some feeling for how things have turned out for me scientifically and for my satisfaction with them. In retrospect, the successes seem greater than I had any right to expect. Nonetheless, I still sometimes doubt the wisdom of my decision to set out on my own, as it were, rather than within a neurobiology department (especially the one here) as you recommended. There is little question that I would have learned more neurobiology faster, but perhaps not so thoroughly, than I have by making all my own mistakes, and there is no question that finding the sort of job I'd like would be easier were I emerging from a highly regarded neurobiology department rather than from a molecular biology lab whose interest in neurobiology is not widely known. On the other hand, I almost certainly would not have come away with the feeling of "owning" an independently developed and promissing system that I now have. In the end, things may turn out so that I'll get some of the best of both; the neurobiology group has been perceptibly warmer since I gave them a seminar on the project last November, and they have apparently

begun to include my name in responses to inquiries from places wanting neurobiologists. If things don't work out at Boulder or Santa Barbara, that should be a help.

Say hi to Naiomi for me. It was a pleasure to see her during her trip East last summer.

Sincerely yours,

Lawrence Okun